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(54) Title: GENETIC LINKAGES BETWEEN AGRONOMICALLY IMPORTANT GENES AND RESTRICTION FRAGMENT LENGTH POLYMORPHISMS

(57) Abstract

The invention concerns methods for finding correlations between agronomically important genes in corn and restriction fragment length polymorphisms and DNA probes shown to reveal polymorphisms.

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TITLE OF THE INVENTION

GENETIC LINKAGES BETWEEN AGRONOMICALLY IMPORTANT GENES AND RESTRICTION FRAGMENT LENGTH POLYMORPHISMS

This is a continuation-in-part of application Serial No. 158,469, filed February 22, 1988.

Field of the Invention

This invention is in the field of genetic engineering and corn breeding.   More specifically, the invention concerns methods for finding correlations between agronomically important genes in corn and restriction fragment length polymorphisms through the use of DNA probes that are shown to reveal polymorphisms.

Background of the Invention

The goal of plant breeding is to combine in a single variety/hybrid various desirable traits of the parental lines and to exploit the heterosis exhibited by the cross of the parental lines. For field crops, these traits may include resistance to diseases and insects, tolerance to heat and drought, reducing the time to crop maturity, greater yield, and better agronomic quality.   With mechanical harvesting of many crops, uniformity of plant

characteristics such as germination and stand establishment, growth rate, maturity and fruit size are important.

Field crops are bred through techniques that use the plant's method of pollination. A plant is defined as self- pollinating if pollen from one flower is transferred to the same or another flower of the same plant. A plant is cross- pollinated if the pollen comes from a flower on a different plant.

Plants that have been self-pollinated and selected for type for many generations become homozygous at almost all gene loci and produce a uniform population of true breeding progeny. A cross between two homozygous plants from differing backgrounds or two homozygous lines (inbred lines) produces a uniform population of hybrid plants that may be heterozygous for many gene loci. A cross of two plants, each heterozygous at a number of gene loci, will produce a population of hybrid plants. Each of these plants differ genetically and the population will not be uniform.

Corn (Zea mays L.) plants are bred by both self- pollination and cross-pollination techniques. Corn has male flowers, located on the tassel, and female flowers, located on the ear, on the same plant. Natural pollination occurs in corn when wind blows pollen from the tassels to the silks that protrude from the tops of the incipient ears.

The development of corn hybrids requires the development of homozygous inbred lines, the crossing of these lines, and the evaluation of the crosses. Pedigree breeding and recurrent selection breeding methods are used to develop inbred lines from breeding populations. Breeding programs are designed to combine desirable traits from two or more inbred lines or various broad-based sources into breeding pools from which new inbred lines are developed by selfing and selection of desirable phenotypes. The new inbreds are crossed with other inbred lines and the hybrids from these crosses are evaluated to determine which have commercial potential as F[1] hybrids.

The identification of desirable agronomic traits has traditionally been done by phenotypic selection. It would be valuable to plant breeders to be able to identify genes affecting agronomic traits on the plant genome through the identification of linked genetic markers. Technology now provides a method for identifying genetic markers with potential application in plant breeding through the use of restriction fragment length polymorphisms (RFLPs). It is therefore of great importance to investigate the possibility of using genetic linkage analysis between DNA polymorphisms and traits of agronomic importance in order to identify agronomically important genes, to classify inbreds, hybrids and breeding populations according to their genes, and to then more effectively incorporate these genes into improved inbreds and hybrids.

Summary of the Invention

The invention is based on the use of RFLPs to identify genetic linkages with agronomically important genes. This invention consists of three major parts: (1) DNA probes shown to reveal polymorphisms between two parent inbred lines and having known chromosomal locations, (2) statistical techniques that can find correlations between the inheritance of one or more DNA probes and the phenotype of the plants under investigation, and (3) methods for using the identified genetic linkage between specific probes and genetic components of agronomically important traits in plant breeding.

In particular, this invention relates to a method for determining a particular trait in a maize plant which comprises analyzing each maize chromosome for DNA polymorphisms linked to a particular trait. Any of a variety of RFLPs, probes and restriction enzymes can be used, as illustrated herein. The invention also relates to specific DNA probes that can be used in the method of this invention. The invention further relates to use of newly-identified relationships

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between agronomic traits and genetic markers to enhance plant breeding.

Detailed Description of the Invention

Definitions

In the description and examples that follow, a number of terms are used herein. In order to provide a clear and consistent understanding of the specification and claims, including the scope to be given such terms, the following definitions are provided:

Restriction Endonuclease. A restriction endonuclease or restriction enzyme is an enzyme that recognizes a specific base sequence in a double-stranded DNA molecule, and will cleave both strands of the DNA molecule at every place where this sequence appears.

Restriction Fragments. The DNA molecules produced by digestion with a restriction endonuclease are referred to as restriction fragments. Any given genome will be digested by a particular restriction endonuclease into a discrete set of restriction fragments. The DNA fragments that result from restriction enzyme cutting are separated and displayed by electrophoresis through agarose gels.

Restriction Fragment Length Polymorphism (RFLP). The genomic DNA of two individuals in a species, for example, will differ in sequence at many sites. When these differences occur in the recognition site for a restriction endonuclease, the enzyme will not cleave the DNA molecule at that point. Likewise, a variation may introduce a recognition site where none exists in the other individual, causing the DNA to be cut by the restriction enzyme at that point. Because of this, digestion of the two individuals' DNA will produce fragments having different lengths. A polymorphism in the length of restriction fragments produced by digestion of the DNA of the two individuals will result.

Agarose Gel Electrophoresis. To detect a polymorphism in the length of restriction fragments, an analytical method for fractioning double-stranded DNA molecules on the basis of size is required. The most commonly used technique for achieving such fractionation is agarose gel electrophoresis. The rate at which DNA fragments move in such gels depends on their size; thus, the distances traveled decrease as the fragment lengths increase.

The DNA fragments fractionated by agarose gel electrophoresis can be visualized directly by a staining procedure if the number of fragments included in the pattern is small. However, most genomes, including the maize genome, contain far too many DNA sequences to produce a simple pattern of restriction fragments. In order to visualize a small subset of these fragments, a methodology referred to as the Southern hybridization procedure can be applied.

Southern Hybridization Procedure. The purpose of the Southern hybridization procedure, also referred to as Southern blotting, is to transfer physically DNA fractionated by agarose gel electrophoresis onto a support such as nylon membrane or nitrocellulose filter paper while retaining the relative positions of DNA fragments resulting from the fractionation procedure. The methodology used to accomplish the transfer from agarose gel to the support is to draw the DNA from the gel into the support by capillary action.

Nucleic Acid Hybridization. Nucleic acid hybridization is used to detected related DNA sequences by hybridization of single-stranded DNA on supports such as nylon membrane or nitrocellulose filter papers. Nucleic acid molecules that have complementary base sequences will reform the double- stranded structure if mixed in solution under the proper conditions. The double-stranded structure will be formed between two complementary single-stranded nucleic acids even if one is immobilized on a support. In the Southern hybridization procedure, the latter situation occurs. As noted previously, the maize genomic DNA is digested with a restriction endonuclease, fractionated by agarose gel electrophoresis, converted to the single stranded form,

and transferred to the support, making it available for reannealing to the hybridization probe.

Hybridization Probe. To detect a particular DNA sequence in the Southern hybridization procedure, a labeled DNA molecule or hybridization probe is reacted to the fractionated DNA bound to a support such as nylon membrane or nitrocellulose filter paper. The areas on the filter that carry DNA sequences complementary to the labeled DNA probe become labeled themselves as a consequence of the reannealing reaction. The areas of the filter that exhibit such labeling can then be detected according to the type of label used. The hybridization probe is generally produced by molecular cloning of a specific DNA sequence from the maize genome.

Description of the Preferred Embodiments

This invention is based on the use of restriction fragment length polymorphism (RFLP) to identify genetic linkages to agronomically important genes. This invention consists of methods for locating agronomically important genes based on RFLPs and of novel DNA probes for use in the methods.

Specifically this invention is based on the identification of restriction fragments from the ten chromosomes of maize that define genetic linkages between specific chromosomes and agronomically important traits using analysis techniques that can find correlations between the inheritance of one or more DNA sequences and the phenotype of the plant under investigation.

Identifying RFLPs involves the use of restriction endonucleases, DNA mapping, and cloned DNA probes. Restriction endonucleases cleave the genomic DNA molecules at specific sites. Cloned RFLPs are detected as the differences in the size of restriction fragments observed in Southern blotting experiments using cloned DNA probes free of repetitive sequences. Certain polymorphisms can thus be genetic markers that are associated with a specific agronomic trait.

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Establishment of such an association permits the monitoring of heritable sequences of genomic DNA. Probes that can detect sequences associated with specific traits can therefore be derived from known gene loci or from anonymous DNA segments. RFLPs appear to be present in all regions of the maize genome, thus making it feasible to construct a detailed maize genetic linkage map and thereby localize agronomically important genes.

RFLPs that identify genetic characteristics are particularly useful in breeding programs in order to select for certain traits. Other uses of RFLPs may include varietal identification, identification and mapping of quantitative trait loci (QTL), quantification of genetic diversity in a crop population, screening genetic resource strains, or populations for useful quantitative trait alleles and their marker-assisted introgression from the resource strain to a commercial variety, and marker-assisted early selection of recombinant inbred lines in plant pedigree breeding programs.

In accordance with the invention, RFLPs are used to identify genetic linkage to agronomically important genes. This invention consists of three major parts: (1) DNA probes shown to reveal polymorphisms between two parent inbred lines and having known chromosomal locations, (2) statistical techniques that can find correlations between the inheritance of one or more DNA probes and the phenotype of the plants under investigation, and (3) use of the identified genetic linkages between specific probes and genetic components of agronomically important traits as an aid in selecting plants and populations in "classical" plant breeding based on Mendelian genetics.

More particularly, the invention comprises determining a particular trait in a maize plant by analyzing maize chromosomes for DNA polymorphisms and linkage to that trait. Specific traits determined include adjusted yield, plant yield, plant height, ear height, GDU shed, GDU silk, grain moisture, root lodging, stalk

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lodging, and stay green. A definition of each of these traits is as follows:

Adjusted Yield, Bushels/Acre. The yield in bushels/acre is the actual yield of the grain at harvest adjusted to 15.5% moisture.

Plant Yield. The plant yield is the field weight divided by the number of plants per plot.

Plant Height. This is a measure of the height of the hybrid from the ground to the tip of the tassel.

Ear Height. The ear height is a measure from the ground to the top ear node attachment.

GDU Shed. The GDU is the number of growing degree units (GDU) required for an inbred line or hybrid to shed pollen from the time of planting. Growing degree units are calculated by the Barger Method, where the heat units for a 24-hour period are:

$$GDU = \frac{(Max. + Min.)}{2} - 50$$

The highest maximum used is 86°F and the lowest minimum used is 50°F. For each hybrid it takes a certain number of GDUs to reach various stages of plant development. GDUs are a way of measuring plant maturity.

Grain Moisture. The grain moisture is the percentage moisture of the grain at harvest.

Root Lodging. The root lodging is the percentage of plants that do not root lodge; i.e., those that lean from the vertical axis at an approximately 30° angle or greater would be counted as root lodged.

Stalk Lodging. This is the percentage of plants that do not stalk lodge, i.e., stalk breakage, as measured by either natural lodging or pushing the stalks and determining the percentage of plants that break off below the ear.

Stay Green. Stay green is the measure of plant health near the time of black layer formation (physiological maturity).

In general, to identify a polymorphism according to this invention, DNA is extracted from the plant cell and digested with a

given restriction endonuclease. After the digest is obtained, and the same is separated by a standard technique such, as, for example, agarose gel electrophoresis, the separated bands are probed with a DNA fragment coding for the RFLP sequence.

Specific probes that may be used in this invention are discussed in the Examples detailed below. Other probes for the polymorphisms can be obtained.

For example, methods for generating additional new DNA fragments also linked with the gene for a particular trait are as follows:

A first method is to test randomly chosen maize DNA fragments (either genomic or c-DNA clones) that map to the appropriate region of the maize genomic map. Such mapping can be achieved by two techniques:

(a) _in_ _situ_ hybridization to metaphase chromosome spreads; or

(b) genetic linkage to any marker as already mapped to the region.

For method, (a), the new fragment need not be polymorphic, but for (b), polymorphisms must first be identified by comparing the restriction pattern of the genomic DNA at the new site in unrelated plants. In method (a), the mapped fragment must still be shown to detect a polymorphism in maize DNA. The polymorphism which represents a genetic marker can then be tested for genetic linkage with genes affecting agronomic traits or can be tested for linkage to other DNA probes, such as those described below.

Another method for obtaining DNA clones is to construct a library from maize DNA isolated from metaphase chromosomes that have been sorted on a fluorescence activated sorter. This method can sometimes yield purified chromosomes of 95% or greater purity.

A final method of obtaining new DNA probes from the region of the chromosome containing the agronomically important gene is to use any probes already mapped to the region in order to "fish out" adjacent overlapping pieces of DNA from genomic libraries (commonly called "chromosome walking").

In all the cases outlined above, a probe must ultimately be found to detect a polymorphism if it is to be useful for testing for the desired trait. The polymorphism must be found to be linked to genes affecting traits or to other useful markers in studies, or to be immediately adjacent to preexisting markers.

The particular probe can be of any desired sequence length as long as it is capable of identifying the polymorphism in the involved DNA region or locus. It can be a DNA fragment by itself, or be present in longer genetic sequences or fragments, or even in a plasmid or other appropriate vehicle. Labelling and hybridization conditions can be readily determined by those of skill in the art. Usually, the stringency is standard for unique sequence DNA from within the species.

A genetic linkage map was constructed from the data presented in the Examples by utilizing the algorithms described by E. Lander et al., Genomics, 1:174-181 (1987), herein incorporated by reference. These genetic linkage groups were assigned to chromosome arms by (1) using as probes DNA sequences whose location was known, and/or (2) using as markers isozyme loci whose location was known.

By applying statistical analysis to maps, markers, and observed traits in field testing, a detailed table has now been constructed which associates specific genetic markers with the loci of genes which influence specific agronomic traits at a high degree of statistical significance (p<0.05), and quantitates the amount of the variation in the observed trait which is accounted for by the genes associated with individual markers. As a result, this invention also provides methods for augmenting conventional plant breeding by identification of individual plants which have the desired genotype at a genetic marker locus associated with one of the foregoing traits, comprising the steps of

- constructing a preferred RFLP profile for genetic markers identified herein as being associated with the trait or traits in question;

- determining the RFLP profiles of individuals in a segregating population with respect to the associated genetic markers; and

- selecting individuals from the population having RFLP profiles which most closely match the preferred profile.

The construction of the preferred RFLP profile is a matter within the skill of the typical plant breeder. For example, the breeder will select a particular allele which provides a desirable contribution in terms of ear circumference. The contribution viewed as "desirable" will vary according to the objectives of the breeding program. In one instance, a large ear circumference be be desirable, while in another, a small ear circumference may be preferred. In yet another instance, the ear circumference _per_ _se_ may be unimportant, but the breeder may be working to develop a plant which is homozygous, i.e., has the same contribution from both parents, with respect to that trait. In any event, it will be a simple matter for the breeder to prepare a list of desired traits and to construct a preferred RFLP profile for the ideal plant from that list and the genetic marker linkages identified herein.

A segregating population of plants is easy to obtain, and is typically found, for example, in the progeny of selfed F_1 hybrids of two different inbred lines. Once the population has been identified, RFLP profiles of each plant are performed using the well-known techniques described above.

Selection of the individuals having RFLP profiles which most closely match the ideal profile simply involves comparison of each individual profile with the ideal profile. Some judgement may be required in this comparison, but the exercise of such judgment will be well within the skill of the typical plant breeder. For example, it will be extremely unlikely, and depending upon the population examined it may be genetically impossible, for any individual to have an RFLP profile which precisely matches the ideal. However, one or more individuals will have a greater percentage of matches to the ideal

than the remainder of the population. Where two individuals have approximately equal matches to the ideal, the breeder will make tradeoffs among traits. One individual may offer the desired allele for ear circumference and kernel row length, but lack the desired allele for stay green. Another individual will offer the desired allele for ear circumference and stay green, but will lack the desired allele for kernel row length. Selection between the two will simply involve a decision by the breeder on whether it is preferable to have the allele for kernel row length or for stay green, and in fact such decisions will be a relatively trivial exercise compared to the judgements made in conventional plant breeding, which are based on less clear-cut information about the genetic makeup of the individuals. The breeder also has the option of proceeding to the next stage of breeding with both individuals.

The following tables identify specific trait-marker linkages for a number of agronomic traits commonly involved in plant breeding. Markers identified by the prefix PIO- are available commercially from Pioneer Hi-Bred International, Des Moines, IA 50309. Markers identified by the prefixes BNL- and UMC- are publicly available markers which can be obtained from Brookhaven National Laboratory and the University of Missouri, respectively. The remainder of the markers are either published isozyme markers of known genetic location, such as AMP1, MDH2 and GLU1, or are probes for specific mutant genes which are well known and whose location has been identified, as mentioned above.

These Tables identify linkages observed in bulk F_4 populations, which reflect inbred performance, and in topcross trials, which reflect performance in hybrids. As might be expected, some overlap is observed between the two sets of linkages.

Traits not self-explanatory and not identified elsewhere herein include:

Grain quality. This is a subjective score based on visual grading of the shelled corn in terms of moldy and cracked kernels.

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Cold test germination. This is a percentage germination test performed in a cold chamber, and evaluates germination performance under adverse conditions.

Soak test germination. This is a test of percentage germination under ideal germination conditions, but after the seed has been soaked prior to planting.

Bulk density, adjusted. This is a measure of weight per unit volume of bulk seed, adjusted to 15.5% moisture.

Dropped ears. This is a raw count of ears found on the ground per two-row test plot.

European corn borer second brood tolerance. This is a visual evaluation of plant damage caused by infestation by the second annual brood of the European corn borer caterpillar.

Plant yield. This is the yield of a test plot divided by the number of plants in the test plot.

Early stand count. This is a raw count of plants per test plot (or number of seeds planted) after emergence and before thinning.

Bare tip. Also called nose back, this is a visual grade based upon the amount of ear tip which is devoid of kernels.

"Statistically transformed" measurements involve the same physical measurements or evaluations as the raw scores, but the raw scores have then been put through a standard statistical transformation to fit them to a normal curve. Unless otherwise indicated, all values measured are averages over the individual ear, plant, test plot or larger field.

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Table 1

List of genetic markers associated with quantitative traits evaluated in topcross progeny.

TRAIT=Yield, Bushels/Acre (BU_ACR)

BNL6.20	PIO20576	PIO20558	PIO20509
PIO20508	PIO20508	BNL5.37-	BNL5.37-
BNL10.24	BNL6.16	BNL7.65	UMC015
PIO2071	PIO20589	PIO20569	PIO1537
PIO20708	PIO20684	PIO20746	UMC116
UMC110	BNL8.32	BNL8.39	PIO20593
PIO20714			

TRAIT= % Moisture (MST)

PIO20640	BNL12.06	UMC061	UMC034
PIO1012	PIO205	PIO2017	BNL6.20
PIO20569	PIO1537	UMC116	BNL8.32
BNL10.39	UMC120	PIO20714	UMC012
PIO2052	PIO20646	PIO1513	

TRAIT=Bulk Density, adjusted (TSTWTA)

PIO20518	PIO1012	PIO205	PIO2017
BNL6.20	PIO20511	PIO20558	PIO20509
PIO20508	PIO20508	BNL7.65	UMC015
PIO2071	PIO1025	PIO612	PIO1524
PIO20589	PIO2045	PIO20569	PIO1537
PIO20708	PIO20684	PIO20746	UMC116
UMC110	BNL8.32	SH1	WX1
PIO2052	UMC081	CSS1	PIO20626
PIO2075	BNL3.04	PIO65	GLU1
PIO20646	PIO1033		

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TRAIT=Yield/% moisture (YLDMST)

BNL12.06	PIO20518	BNL8.29	UMC061
UMC034	PIO1012	PIO20576	PIO20558
PIO20509	PIO20508	PIO20508	BNL5.37-
BNL5.37-	BNL10.24	BNL6.16	UMC066
UMC019	BNL7.65	PIO2071	BNL8.33
UMC043	PIO20622	PIO612	PIO1524
PIO20589	PIO2026P		

TRAIT=GDU Shed (GDUSHD)

PIO1012	PIO205	PIO2017	PIO20576
PIO20558	PIO20509	PIO20508	PIO20508
BNL5.37-	BNL5.37-	BNL10.24	PIO20521
PIO1533	UMC999	PIO20713	PIO20725
BNL5.46	PIO612	PIO1524	PIO20589
BNL13.05	PIO1040	BNL10.39	UMC120
PIO20714	UMC012	WX1	PIO2052
UMC081	PIO20554	BNL10.13	

TRAIT=Stay green (STAGRN)

PIO20690	PIO2017	BNL6.20	BNL5.37-
BNL10.24	UMC046	MDH2	PIO20595
PIO20599	PIO20569	PIO1537	PIO20684
UMC116	BNL8.32	BNL10.39	UMC120
PIO20714	UMC012	SH1	BNL3.04

TRAIT=Dropped Ears (DRPEAR)

BNL10.39	UMC081	BNL7.21B	BNL10.13

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<u>TRAIT=Dropped Ears, Statistically transformed (FOGDE)</u>
PI020690

<u>TRAIT=Ear height (EARHT)</u>

PI020640	UMC034	PI020576	PI020511
PI020558	PI020509	PI020508	PI020508
BNL5.37-	BNL5.37-	BNL10.24	PI020521
PI01533	PI020713	PI020725	BNL8.33
PI020569	BNL8.32	BNL8.39	PI020593
BNL10.39	UMC120	PI020714	UMC012
WX1	UMC081	PI020646	PI01513

<u>TRAIT=Plant height (PLTHT)</u>

PI020690	PI020668	PI020518	PI020576
PI020511	PI020558	PI020509	PI020508
PI020508	BNL5.37-	BNL5.37-	BNL10.24
BNL6.16	PI020521	PI01533	UMC999
PI01080	BNL8.33	PI01040	BNL10.39
UMC120	UMC012	PI065	GLU1
PI020646	PI01513	UMC044	

<u>TRAIT=Grain quality (GRNQUL)</u>

BNL12.06	BNL8.29	BNL6.20	UMC999
BNL6.29	PI020528	PI020599	BNL14.28

<u>TRAIT=European Corn Borer Second Brood Tolerance (ECB2SC)</u>

PI02017	PI020576	PI020509	PI020508
BNL5.37-	PI020569	PI01537	PI020708
UMC116	BNL8.39	PI020593	BNL10.39
UMC120	PI020626	PI02075	

TRAIT=Root lodging (RT LDG)

UMC094	PI020511	BNL5.37-	BNL5.37-
BNL10.24	BNL6.16	UMC999	BNL8.23
BNL6.29	PI020527	PI020528	PI020854
PI02045	PI067	UMC065	MDH2
PI020595	UMC012	PI020562	PI020646

TRAIT=Root lodging, statistically transformed (FOGRTL)

UMC094	PI020576	PI020511	BNL5.37-
BNL5.37-	BNL10.24	UMC999	UMC019
BNL6.29	PI020527	PI020528	PI020854
PI02045	PI067	UMC065	MDH2
PI020595	UMC012	PI020562	

TRAIT=Stalk lodging (STKLDG)

PI020622	PI01537	BNL14.28	PI020562
UMC057	UMC044		

TRAIT=Stalk lodging, statistically transformed (FOGSTL)

PI020537	PI01012	PI0205	E8
PI020558	PI020597	PI020622	PI0612
PI01524	UMC065	PI01537	UMC110
PI020626	PI02075	PI020646	PI01513
UMC057	BNL10.13	UMC044	

<u>Table 2</u>

List of genetic markers associated with quantitative traits as identified in bulk f4 progeny

<u>TRAIT=Yield, Bushels/Acre (BU ACR)</u>

BNL5.59	BNL6.20	UMC042	PIO20608
UMC043	PIO20622	PIO612	PIO1524
PIO1518	PIO20589	PIO105	SH1
PIO1513			

<u>TRAIT=Plant yield (PLNTYLD)</u>

PIO20537	PIO20518	BNL8.29	PIO20557
BNL6.20	UMC043	PIO20622	PIO1524
PIO1518	PIO20589	PIO20523	PIO20569
PIO105	SH1	PIO20626	PIO20646

<u>TRAIT=Weight of 100 kernels (CKWT)</u>

PIO20603	PIO20689	PIO20654	BNL5.59
PIO20682	PIO20674	PIO20575	PIO20644
BNL8.23	PIO67	UMC065	UMC021
UMC110	PIO20714	PIO105	PIO20554
BNL14.28	UMC057	BNL10.13	UMC044

<u>TRAIT=Dropped ears, statistically transformed (FOGDE)</u>

PIO205	PIO2017	PIO20558	PIO20508
UMC066	UMC019	PIO1518	PIO20531
PIO20527	PIO20528	PIO67	BNL8.39
PIO20593			

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TRAIT=Cold test germination (CTST)

UMC034	BNL8.33	PIO1017	PIO67
UMC065	UMC021	PIO20569	PIO1537
PIO20708	PIO20684	PIO20746	UMC116
BNL8.32	BNL8.39	SH1	PIO65
GLU1	PIO20646		

TRAIT=Soak test germination (SKTST)

PIO20518	PIO20511	PIO20509	BNL8.33
PIO20569	PIO20708	PIO20746	BNL9.11
PIO2052	GLU1		

TRAIT=Early stand count (ESTCNT)

UMC061	PIO20713	PIO20725	PIO20597
UMC042	BNL7.65	BNL8.33	PIO1016
PIO65			

TRAIT=Ear circumference (EARCIR)

PIO20603	PIO20640	BNL6.20	PIO20508
BNL5.37-	BNL10.24	UMC999	PIO20726
UMC031	BNL5.46	PIO20597	UMC042
UMC019	BNL7.65	UMC015	PIO2071
PIO1025	PIO20608	PIO20622	PIO612
PIO1524	PIO1518	PIO20589	PIO20531
PIO2026P	PIO67	PIO20595	PIO20599
PIO20569	PIO1537	PIO20708	PIO20746
UMC116	BNL10.39	UMC120	PIO105
UMC081	PIO65		

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TRAIT=Ear diameter (EARDIA)

PIO20509	PIO20508	PIO20508	BNL5.37-
BNL10.24	UMC999	PIO20726	UMC031
PIO2071	PIO1025	PIO20622	PIO612
PIO1518	PIO20595	PIO20599	PIO20569
PIO1537	PIO20708	PIO20746	BNL8.39
UMC081			

TRAIT=Ear length (EARLGT)

PIO20557	PIO20726	BNL15.45	UMC015
PIO2071	PIO20854	UMC062	BNL8.39
PIO2020	PIO1040	PIO20554	

TRAIT=Cob circumference (COBCIR)

PIO20640	BNL12.06	BNL8.29	PIO20557
BNL6.20	BNL5.37-	UMC999	PIO1080
PIO20726	UMC031	BNL5.46	PIO20597
BNL15.45	UMC042	UMC066	UMC019
BNL7.65	UMC015	PIO2071	PIO1025
PIO20608	UMC043	PIO20622	PIO612
PIO1524	PIO1518	PIO20589	PIO20531
PIO2026P	UMC065	UMC081	PIO65

TRAIT=Cob diameter (COBDIA)

PIO20640	BNL12.06	PIO20713	PIO20725
UMC031	BNL5.46	PIO20597	BNL15.45
UMC042	UMC066	UMC019	BNL7.65
UMC015	PIO2071	PIO1025	PIO20608
UMC043	PIO20622	PIO612	PIO1524
PIO1518	PIO20589	PIO20531	PIO2026P
UMC065	UMC021	UMC046	UMC062
MDH2	PIO20599	BNL8.44	UMC081
PIO65	GLU1	PIO20646	

TRAIT=Bare tip (NOSBAC)

PIO20640	PIO20644	PIO20661	PIO2017
BNL10.24	BNL15.45	UMC042	UMC066
PIO1025	PIO612	PIO1524	PIO1518
PIO20589	PIO20531	PIO2026P	PIO20854
UMC065	PIO20569	PIO20708	UMC116
BNL10.39	UMC012	WX1	PIO2052
UMC081	CSS1	PIO20554	PIO2075

TRAIT=Kernel row length (ROWLEN)

PIO20518	BNL8.29	PIO20557	PIO20726
UMC015	PIO2071	PIO1524	PIO20523
PIO20527	UMC065	UMC062	UMC116
PIO1040	SH1		

TRAIT=Kernel Depth (KDEPTH)

PIO20603	PIO20640	PIO20661	PIO1012
PIO2017	E8	PIO20622	UMC021
PIO20569	PIO1537	PIO20708	UMC110
BNL8.39			

TRAIT=Kernels per kernel row (KERPRO)

BNL12.06	UMC061	BNL6.20	E8
PIO20713	PIO20725	UMC031	UMC043
PIO20622	PIO612	PIO1524	PIO1518
PIO20589	UMC065	PIO1014	UMC062
BNL10.39	UMC081	CSS1	PIO20554
PIO20626			

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TRAIT=Number of kernel rows per ear (KERROW)

PIO20690	UMC999	PIO20726	UMC031
BNL5.46	PIO20597	UMC042	UMC019
BNL7.65	UMC015	PIO2071	PIO1025
PIO20608	PIO612	PIO1524	PIO1518
PIO20589	PIO20531	PIO20566	PIO2026P
PIO2045	UMC065	UMC046	PIO20569
PIO1537	PIO20708	PIO20684	PIO20746
UMC116	UMC110	BNL8.32	BNL8.44
PIO105	UMC057	UMC044	

TRAIT=Kernel width (KWIDTH)

BNL12.06	UMC023	PIO20726	UMC015
PIO2071	PIO20608	PIO612	PIO1524
PIO20589	PIO20531	PIO20566	PIO2026P
PIO2045	UMC065	PIO20569	PIO1537
PIO20708	PIO20684	PIO20746	UMC116
UMC110	BNL8.32	BNL8.44	UMC012
UMC057	BNL10.13	UMC044	

TRAIT=% Moisture (MST)

UMC094	PIO20537	PIO20603	PIO20689
PIO20640	PIO20575	PIO2044	PIO20661
PIO20518	BNL8.29	PIO20557	UMC061
UMC043	PIO20622	PIO20531	UMC021
PIO1016	BNL8.32	PIO20593	BNL10.39
PIO65	PIO1513	UMC057	BNL10.13
UMC044			

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<u>TRAIT=Yield/% moisture (YLDMST)</u>

BNL12.06	BNL5.59	UMC061	BNL6.20
BNL6.16	UMC042	BNL7.65	PIO20608
UMC043	PIO20622	PIO612	PIO1524
PIO1518	PIO20589	PIO20527	PIO105
SH1	PIO20646	PIO1513	

<u>TRAIT=GDU Shed (GDUSHD)</u>

PIO20640	BNL12.06	UMC034	PIO1012
PIO205	BNL5.37-	BNL5.37-	BNL10.24
PIO20713	PIO20725	BNL5.46	UMC042
UMC066	PIO20854	BNL13.05	BNL9.11
UMC012	WX1	PIO2052	UMC081
CSS1	PIO20554	BNL8.17	BNL7.21B
GLU1	PIO20646	PIO1513	PIO1033
UMC057	BNL10.13		

<u>TRAIT=GDU Silk (GDUSLK)</u>

PIO20690	PIO20537	PIO20640	BNL10.24
PIO20713	PIO20725	BNL5.46	UMC042
UMC066	PIO1524	PIO20854	BNL13.05
UMC012	PIO105	SH1	WX1
PIO2052	UMC081	CSS1	PIO20554
BNL8.17	GLU1	PIO20646	PIO1513
BNL10.13			

<u>TRAIT=Stay green (STAGRN)</u>

PIO20640	BNL12.06	PIO20654	BNL5.59
PIO20682	PIO20674	PIO20575	PIO20644
PIO2044	PIO20661	PIO20557	UMC034
BNL6.16	PIO1518	PIO20531	PIO20566

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PIO20527	BNL8.39	BNL8.44	PIO2020
WX1	BNL8.17	UMC057	BNL10.13
UMC044			

TRAIT=Plant height (PLTHT)

BNL12.06	UMC034	PIO1012	PIO205
PIO20576	PIO20508	BNL5.37-	BNL5.37-
BNL10.24	PIO20713	PIO20725	PIO20728
BNL10.39	UMC120	UMC012	WX1
PIO2052	UMC081	PIO20554	PIO20626
PIO2075	GLU1	PIO20646	

TRAIT=Ear height (EARHT)

PIO20640	BNL12.06	PIO2044	UMC034
PIO1012	PIO205	PIO20576	PIO20509
PIO20508	PIO20508	BNL5.37-	BNL5.37-
BNL10.24	BNL6.16	PIO20521	UMC999
PIO20713	PIO20725	BNL6.29	PIO20728
BNL10.39	UMC120	UMC012	WX1
PIO2052	UMC081	PIO20554	UMC057

TRAIT=European Corn Borer Second Brood Tolerance (ECB2SC)

BNL5.59	PIO20644	PIO205	BNL6.20
E8	BNL6.16	PIO20597	BNL15.45
UMC042	PIO20608	PIO612	PIO2045
UMC065	MDH2	PIO20595	PIO20599
PIO20569	PIO1537	PIO20708	UMC110
BNL10.39	UMC120	PIO105	

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TRAIT=Root lodging (RT_LDG)

UMC094	PI020537	PI020603	PI020689
PI020640	PI020518	PI020576	PI020511
PI020558	PI020509	PI020508	PI020508
BNL5.37-	BNL10.24	PI020713	PI020725
UMC031	UMC066	UMC019	UMC065
MDH2	PI020595	UMC081	

TRAIT=Root lodging, statistically transformed (FOGRTL)

PI020640	PI020654	PI020644	PI020518
PI020576	PI020511	PI020558	PI020509
PI020508	PI020508	BNL5.37-	BNL5.37-
BNL10.24	UMC019	BNL6.29	UMC065
PI020581	UMC081		

TRAIT=Stalk lodging (STKLDG)

BNL6.20	PI020597	MDH2	PI020595
PI020599	PI020728	UMC012	PI0105
PI02075	UMC057		

TRAIT=Stalk lodging, statistically transformed (FOGSTL)

PI020682	PI020661	PI020668	AMP1
BNL6.20	UMC042	PI067	PI020595
PI020599	BNL13.05	PI0105	SH1
BNL3.04			

TRAIT=Grain quality (GRNQUL)

BNL5.59	PI020575	PI020644	PI02044
PI020661	AMP1	PI020557	UMC061
PI01012	PI0205	PI02017	E8
PI020509	PI02071	PI01025	BNL8.33
PI020569	PI01537	PI020708	PI020684
PI020746	UMC116	UMC110	BNL8.32

BNL8.39	PIO20593	PIO1033	UMC057
BNL10.13			

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Having now defined the invention, the same will be understood by means of specific examples which are, however, not intended to be limiting unless otherwise specified.

EXAMPLE 1

Determination of Agronomically Useful Phenotypes

Development of Progenies for Field Testing

The inbreds B73 and Mo17 were crossed to produce the F_1 hybrid designated B73/Mo17. Hybrid seed was planted, several plants selfed, and the seed bulked to produce the F2 generation designated B73/Mo17)X. 175 seeds from the F2 generation were planted in peat pots in a greenhouse. Seedlings were transplanted to the field at normal planting time. Each plant was self-pollinated using the usual procedures for pollination of corn to produce the F3 ears designated B73/Mo17)Xn where n ranged from 1 to 175 and represents the specific F3 ear. Ears were harvested, identified to plant number, and kept separate. Each ear therefore contained seed that would generate an F3 family.

A winter nursery was used to produce seed for field testing from the F3 ears. Twenty-four kernels from each of 112 F3 ears were planted in an isolated crossing block. Interplanted around those 112 rows were rows of Pioneer inbred V78. Tassels were removed by hand from every plant of the 112 F3 families. Thus, the ear of each F3 plant from the B73/Mo17 cross was pollinated by the inbred V78. For each of the 112 F3 families, each ear from the 24 plants were harvested, dried, shelled, and bulked together. These 112 entities of seed were considered as 112 F3 topcrosses to inbred V78.

In addition, a separate 24 kernels from each F3 ear were planted in 112 rows, one for each F3 family. Within each row, ten plants were self-pollinated to derive F4 ears. For each of the 112 F3 families, each of the self-pollinated ears were harvested, dried, shelled, and

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bulked together. These 112 entities of seed were considered as 112 F4 bulks.

Determination of Whole Plant Phenotype

The 112 F3 topcrosses and 112 F4 bulks were evaluated in field performance tests (yield tests). The tests were conducted according to standard yield test procedures as used in the profession of agronomy and crop breeding.

Each of the 112 F3 topcrosses comprised an entry in a randomized complete block design. Check entries were added making a total entry list of 125. The experiment was grown in two replications at each of four locations in central Iowa. Each replication of an entry was planted in a two-row plot. Plots were 5.3 meters long with .76 meters between rows. Plots were overplanted and thinned to even and uniform stand of 50 plants per plot. These plants were allowed to grow to maturity and data were collected for various traits throughout the season.

The 112 F4 bulks were tested identically except there were three test locations instead of four. Two locations were in central Iowa, and the other was in central Indiana.

The following traits were measured on each plot. Each is considered an agronomic trait important in corn breeding.

TRAIT	ABBREVIATION	DESCRIPTION
Adjusted Yield	BUACR	Field weight adjusted to 15.5% moisture expressed as bushels per acre.
Plant Yield	PLNTYLD	Field weight divided by the number of plants per plot.
Plant Height	PLTHT	From ground to tip of tassel.
Ear Height	EARHT	From ground to top ear node.

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GDU Shed	GDUSHD	Accumulated heat units to the day that 40% of plants in a plot were shedding pollen.
GDU Silk	GDUSLK	Accumulated heat units to the day that 50% of plants in a plot had silks emerged at least one inch.
Grain Moisture	MST	Percent moisture in grain.
Root Lodging	RTLDG	Number of plants per plot leaning from vertical more than 30°.
Stalk Lodging	STKLDG	Number of plants with stalks broken below the ear at harvest.
Stay green	STAGRN	Relative amount of green leaf tissue remaining at physiological maturity.

The data from plots of F3 topcrosses and F4 bulks were made available for statistical analyses in October 1987. Data were collected on the progenies rather than the original, individual F2 plants because of the well-established principle that the heritability of these complex traits is very low when measured on a single plant basis. Each trait is probably governed by more than one gene, and expression is affected by environmental conditions. Thus, by testing F3 topcrosses and F4 bulks in replicated trials, a more accurate measure of phenotype was obtained. These measures a phenotype of F3 and F4 progeny were considered accurate estimates of the phenotype of each 112 F2 plants from which they were derived.

EXAMPLE 2

Identification of Informative Probes

Maize DNA Isolations

Total DNA can be isolated from various maize tissues (leaves, seedlings, etc.) by any one of several standard methods (for example see Maniatis et al., Molecular Cloning, A Laboratory Manual (1982); Dillon et al., Recombinant DNA Methodology (John Wiley & Sons 1985).

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Southern Hybridization

Agarose gel electrophoresis of restriction enzyme- generated fragments of maize DNA, transfer of the DNA to nylon membranes, and hybridization with a radioactively labeled probe can be done by any of several standard methods (for example, see Maniatis et al., Molecular Cloning, A Laboratory Manual).

Identification of Informative DNA Probes

Total DNA from the maize inbred B73 was purified and digested to completion with the restriction enzyme Pst I and electrophoresed on an agarose gel. Fragments from the size classes 600-1,000 base pairs (bp), 1,000-1,500 bp, 1,500-2,000 bp, and 2,000-2,500 bp were ligated into one of several E. coli vectors at the Pst I site and transformed into one of several laboratory strains of E. coli using standard conditions (for example, see Maniatis et al., Molecular Cloning, A Laboratory Manual). Colonies containing plasmids with single inserts were identified by plasmid minipreps and agarose gel electrophoresis.

Plasmid DNA from each previously characterized colony was purified, digested to completion with Pst I, and the two fragments (maize DNA insert and cloning vector) separated by agarose gel electrophoresis. Isolated maize insert DNA, still in the agarose plug, was radioactively labeled with P^{32} by either nick translation or random priming.

Southern hybridization was done using labeled maize insert DNA as probe and total cell DNA from various maize inbreds digested to completion with one or more restriction enzymes as target. After washing the membrane to remove any nonhybridized probe, the membranes were subjected to autoradiography.

Probes were selected for further consideration if (1) 1-3 autoradiographic bands were observed for at least one restriction enzyme, and (2) band patterns were different between at least two maize inbreds.

EXAMPLE 3
Determination of Linkage Among RFLP Marker Loci

Individual probes were hybridized to DNAs prepared from each of the 112 F2 plants described above. Each plant was scored for its allelic composition at the locus defined by the probe. Scores were A (only band(s) contributed by B73 present), B (only band(s) contributed by Mo17 present) or H (bands from both parents present). A genetic linkage map was constructed from these data by using the algorithms described by Lander et al. (1987), supra.

These genetic linkage groups were assigned to chromosome arms by (1) using as probes DNA sequences whose location was known, and/or (2) using as markers isozyme loci whose location was known.

EXAMPLE 4
Determination of Correlations between Phenotypic
Expression of Traits and Specific Probes

Assessment of the Quality of Quantitative Trait Data Obtained in the Field and Determination of Quantitative Trait Data to Group for Investigation Linkages between RFLP Marker Loci and Quantitative Trait Loci

Analysis of variance due to environments, genotypes, and genotype by environment interactions were determined for all quantitative traits. A maximum/minimum test for heterogeneity of variances among locations was used to make sure that data from all locations had similar variability. Data from traits which showed significant variability among genotypes and were highly heritable (> 0.65) were kept for analyses of associations with RFLP marker loci. Because all traits showed some genotype by environment interactions, it was decided to investigate relationships between probes and quantitative traits by location.

Linear and Non-linear Relationships between Quantitative Traits and RFLP Marker Loci Were Used to Detect Linkages between Marker Loci and Quantitative Trait Loci (QTLs).

Data from each test were analyzed using a two-factor analysis of variance for each pair-wise combination of quantitative trait and marker locus; where factors were the marker locus and environment. Data from traits which showed no significant locus by environment interactions, as judged by an F-test, were investigated across environments. Data from traits which showed significant locus by environment interactions were investigated by environment. An F-test was used to determine if significant variability in the expression of a trait was associated with differences in genotypes of a RFLP marker locus. The marker locus is considered linked to a QTL if there is a significant F-value for the variability at the marker locus and either the linear or non-linear orthogonal contrasts associated with the marker.

Based upon the above criteria, loci involved in the expression of grain moisture, adjusted yield, stalk lodging, root lodging, plant height, ear height, plant yield, GDU shed, GDU silk, and stay green in F3 top crosses (TC) and F4 bulk tests (PS) are found to be linked with the indicated mapped probes (Table 3). The numbers in each TC or PS column give the percent of the total variation for that trait expressed in the population associated with a particular RFLP locus. Only values significant at the 0.05 level are shown. CM refers to spacing between the probes in centi-Morgans.

Having now fully described the invention, it will be understood that the same can be carried out within a broad and equivalent range of probes, conditions, enzymes, detection techniques, and the like without affecting the spirit or scope of the invention or of any embodiment herein.

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WHAT IS CLAIMED IS:

1.   A method of determining a correlation between a phenotypic trait in maize and a restriction fragment length polymorphism comprising:

(a) digesting genomic DNA from a maize plant with a restriction endonuclease that produces a restriction fragment length polymorphism digestion pattern that is associated with said trait;

(b) separating the fragments obtained from said digestion in step (a);

(c) detecting said restriction fragment length polymorphism with a hybridization probe containing sequence information capable of hybridizing to and identifying said RFLP, thereby generating a restriction pattern; and

(d) correlating the presence or absence of said RFLP in said digest with the respective presence or absence of said trait.

2.   The method of claim 1 wherein said trait is selected from the group consisting of adjusted yield, plant yield, plant height, ear height, GDU shed, GDU silk, grain moisture, root lodging, stalk lodging, and stay green.

3.   The method of claim 1 wherein said maize plant is maize inbred B73 or maize inbred Mo17.

4.   The method of claim 1 wherein said maize plant is a cross between B73 and Mo17.

5.   The method of claim 1 wherein said hybridization probe is a genomic DNA probe made from maize inbred B73 DNA digested with restriction endonuclease PST I.

6.   A method for identifying and mapping quantitative trait loci (QTL) for a phenotypic trait in maize plant B73, Mo17 or a cross

thereof by analyzing the chromosomes of said plants for DNA polymorphisms and linkage to said trait comprising:

(a) digesting genomic DNA from a maize plant with a restriction endonuclease that produces a restriction fragment length polymorphism digestion pattern that is associated with said trait;

(b) separating the fragments obtained from said digestion in step (a);

(c) detecting said restriction fragment length polymorphism with a hybridization probe containing sequence information capable of hybridizing to and identifying said RFLP, thereby generating a restriction pattern; and

(d) correlating the presence or absence of said RFLP in said digest with the respective presence or absence of said trait wherein said phenotypic trait is selected from the group consisting of adjusted yield, plant yield, plant height, ear height, GDU shed, GDU silk, grain moisture, root lodging, stalk lodging, and stay green.

7. A restriction fragment length polymorphism probe that can detect a polymorphism in one or more maize plants wherein said polymorphism is linked to a phenotypic trait selected from the group consisting of adjusted yield, plant yield, plant height, ear height, GDU shed, GDU silk, grain moisture, root lodging, stalk lodging, and stay green.

8. The probe of claim 7 wherein said maize plant is inbred maize B73, Mo17 or a cross thereof.

9. A method for identifying individual corn plants which have the desired genotype at at least one selected genetic marker locus associated with a desired trait, comprising the steps of

constructing a preferred RFLP profile for each selected genetic marker associated with the desired trait;

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determining the RFLP profiles of individual plants in a segregating population of plants versus the selected genetic markers; and

selecting individual plants which have RFLP profiles which most closely match the preferred RFLP profile.

10. A method according to Claim 9 for identifying individual corn plants which have the desired genotype at a genetic marker locus associated in inbred performance with yield in bushels per acre, comprising the steps of

constructing a preferred RFLP profile with respect to one or more genetic markers selected from BNL5.59, BNL6.20, UMC042, PIO20608, UMC043, PIO20622, PIO612, PIO1524, PIO1518, PIO20589, PIO105, SH1, and PIO1513;

determining the RFLP profiles of individual corn plants in a segregating population of corn plants with respect to the selected genetic marker(s); and

selecting individual plants which have RFLP profiles which most closely match the preferred profile.

11. A method according to Claim 9 for identifying individual corn plants which have the desired genotype at a genetic marker locus associated in inbred performance with plant yield, comprising the steps of

constructing a preferred RFLP profile with respect to one or more genetic markers selected from PIO20537, PIO20518, BNL8.29, PIO20557, BNL6.20, UMC043, PIO20622, PIO1524, PIO1518, PIO20589, PIO20523, PIO20569, PIO105, SH1, PIO20626, PIO20646, and PIO1513;

determining the RFLP profiles of individual corn plants in a segregating population of corn plants with respect to the selected genetic marker(s); and

selecting individual plants which have RFLP profiles which most closely match the preferred profile.

12. A method according to Claim 9 for identifying individual corn plants which have the desired genotype at a genetic marker locus associated in inbred performance with the weight of 100 kernels, comprising the steps of

constructing a preferred RFLP profile with respect to one or more genetic markers selected from PIO20603, PIO20689, PIO20654, BNL5.59, PIO20682, PIO20674, PIO20575, PIO20644, BNL8.23, PIO67, UMC065, UMC021, UMC110, PIO20714, PIO105, PIO20554, BNL14.28, UMC057, BNL10.13, and UMC044;

determining the RFLP profiles of individual corn plants in a segregating population of corn plants with respect to the selected genetic marker(s); and

selecting individual plants which have RFLP profiles which most closely match the preferred profile.

13. A method according to Claim 9 for identifying individual corn plants which have the desired genotype at a genetic marker locus associated in inbred performance with statistically transformed dropped ear count, comprising the steps of

constructing a preferred RFLP profile with respect to one or more genetic markers selected from PIO205, PIO2017, PIO20558, PIO20508, UMC066, UMC019, PIO1518, PIO20531, PIO20527, PIO20528, PIO67, BNL8.39, and PIO20593;

determining the RFLP profiles of individual corn plants in a segregating population of corn plants with respect to the selected genetic marker(s); and

selecting individual plants which have RFLP profiles which most closely match the preferred profile.

14. A method according to Claim 9 for identifying individual corn plants which have the desired genotype at a genetic marker locus

associated in inbred performance with cold test germination, comprising the steps of

constructing a preferred RFLP profile with respect to one or more genetic markers selected from UMC034, BNL8.33, PIO1017, PI067, UMC065, UMC021, PIO20569, PIO1537, PIO20708, PIO20684, PIO20746, UMC116, BNL8.32, BNL8.39, SH1, PI065, GLU1, and PIO20646;

determining the RFLP profiles of individual corn plants in a segregating population of corn plants with respect to the selected genetic marker(s); and

selecting individual plants which have RFLP profiles which most closely match the preferred profile.

15. A method according to Claim 9 for identifying individual corn plants which have the desired genotype at a genetic marker locus associated in inbred performance with soak test germination, comprising the steps of

constructing a preferred RFLP profile with respect to one or more genetic markers selected from PIO20518, PIO20511, PIO20509, BNL8.33, PIO20569, PIO20708, PIO20746, BNL9.11, PIO2052, and GLU1;

determining the RFLP profiles of individual corn plants in a segregating population of corn plants with respect to the selected genetic marker(s); and

selecting individual plants which have RFLP profiles which most closely match the preferred profile.

16. A method according to Claim 9 for identifying individual corn plants which have the desired genotype at a genetic marker locus associated in inbred performance with early stand count, comprising the steps of

constructing a preferred RFLP profile with respect to one or more genetic markers selected from UMC061, PIO20713, PIO20725, PIO20597, UMC042, BNL7.65, BNL8.33, PIO1016, and PI065;

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determining the RFLP profiles of individual corn plants in a segregating population of corn plants with respect to the selected genetic marker(s); and

selecting individual plants which have RFLP profiles which most closely match the preferred profile.

17. A method according to Claim 9 for identifying individual corn plants which have the desired genotype at a genetic marker locus associated in inbred performance with ear circumferenc, comprising the steps of

constructing a preferred RFLP profile with respect to one or more genetic markers selected from PIO20603, PIO20640, BNL6.20, PIO20508, BNL5.37-, BNL10.24, UMC999, PIO20726, UMC031, BNL5.46, PIO20597, UMC042, UMC019, BNL7.65, UMC015, PIO2071, PIO1025, PIO20608, PIO20622, PIO612, PIO1524, PIO1518, PIO20589, PIO20531, PIO2026P, PIO67, PIO20595, PIO20599, PIO20569, PIO1537, PIO20708, PIO20746, UMC116, BNL10.39, UMC120, PIO105, UMC081, and PIO65;

determining the RFLP profiles of individual corn plants in a segregating population of corn plants with respect to the selected genetic marker(s); and

selecting individual plants which have RFLP profiles which most closely match the preferred profile.

18. A method according to Claim 9 for identifying individual corn plants which have the desired genotype at a genetic marker locus associated in inbred performance with ear diameter, comprising the steps of

constructing a preferred RFLP profile with respect to one or more genetic markers selected from PIO20508, PIO20508, BNL5.37-, BNL10.24, UMC999, PIO20726, UMC031, PIO2071, PIO1025, PIO20622, PIO612, PIO1518, PIO20595, PIO20599, PIO20569, PIO1537, PIO20708, PIO20746, BNL8.39, and UMC081;

determining the RFLP profiles of individual corn plants in a segregating population of corn plants with respect to the selected genetic marker(s); and

selecting individual plants which have RFLP profiles which most closely match the preferred profile.

19. A method according to Claim 9 for identifying individual corn plants which have the desired genotype at a genetic marker locus associated in inbred performance with ear length, comprising the steps of

constructing a preferred RFLP profile with respect to one or more genetic markers selected from PIO20557, PIO20726, BNL15.45, UMC015, PIO2071, PIO20854, UMC062, BNL8.39, PIO2020, PIO1040, and PIO20554;

determining the RFLP profiles of individual corn plants in a segregating population of corn plants with respect to the selected genetic marker(s); and

selecting individual plants which have RFLP profiles which most closely match the preferred profile.

20. A method according to Claim 9 for identifying individual corn plants which have the desired genotype at a genetic marker locus associated in inbred performance with cob circumference, comprising the steps of

constructing a preferred RFLP profile with respect to one or more genetic markers selected from PIO20640, BNL12.06, BNL8.29, PIO20557, BNL6.20, BNL5.37-, UMC999, PIO1080, PIO20726, UMC031, BNL5.46, PIO20597, BNL15.45, UMC042, UMC066, UMC019, BNL7.65, UMC015, PIO2071, PIO1025, PIO20608, UMC043, PIO20622, PIO612, PIO1524, PIO1518, PIO20589, PIO20531, PIO2026P, UMC065, UMC081, and PIO65;

determining the RFLP profiles of individual corn plants in a segregating population of corn plants with respect to the selected genetic marker(s); and

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selecting individual plants which have RFLP profiles which most closely match the preferred profile.

21. A method according to Claim 9 for identifying individual corn plants which have the desired genotype at a genetic marker locus associated in inbred performance with cob diameter, comprising the steps of

constructing a preferred RFLP profile with respect to one or more genetic markers selected from PIO20640, BNL12.06, PIO20713, PIO20725, UMC031, BNL5.46, PIO20597, BNL15.45, UMC042, UMC066, UMC019, BNL7.65, UMC015, PIO2071, PIO1025, PIO20608, UMC043, PIO20622, PIO612, PIO1524, PIO1518, PIO20589, PIO20531, PIO2026P, UMC065, UMC021, UMC046, UMC062, MDH2, PIO20599, BNL8.44, UMC081, PIO65, GLU1, and PIO20646;

determining the RFLP profiles of individual corn plants in a segregating population of corn plants with respect to the selected genetic marker(s); and

selecting individual plants which have RFLP profiles which most closely match the preferred profile.

22. A method according to Claim 9 for identifying individual corn plants which have the desired genotype at a genetic marker locus associated in inbred performance with bare tip, comprising the steps of

constructing a preferred RFLP profile with respect to one or more genetic markers selected from PIO20640, PIO20644, PIO20661, PIO2017, BNL10.24, BNL15.45, UMC042, UMC066, PIO1025, PIO612, PIO1524, PIO1518, PIO20589, PIO20531, PIO2026P, PIO20854, UMC065, PIO20569, PIO20708, UMC116, BNL10.39, UMC012, WX1, PIO2052, UMC081, CSS1, and PIO20554;

determining the RFLP profiles of individual corn plants in a segregating population of corn plants with respect to the selected genetic marker(s); and

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selecting individual p lants which have RFLP profiles which most closely match the preferred profile.

23. A method according to Claim 9 for identifying individual corn plants which have the desired genotype at a genetic marker locus associated in inbred performance with kernel row length, comprising the steps of

constructing a preferred RFLP profile with respect to one or more genetic markers selected from PIO20518, BNL8.29, PIO20557, PIO20726, UMC015, PIO2071, PIO1524, PIO20523, PIO20527, UMC065, UMC062, UMC116, PIO1040, and SH1;

determining the RFLP profiles of individual corn plants in a segregating population of corn plants with respect to the selected genetic marker(s); and

selecting individual plants which have RFLP profiles which most closely match the preferred profile.

24. A method according to Claim 9 for identifying individual corn plants which have the desired genotype at a genetic marker locus associated in inbred performance with kernel depth, comprising the steps of

constructing a preferred RFLP profile with respect to one or more genetic markers selected from PIO20603, PIO20640, PIO20661, PIO1012, PIO2017, E8, PIO20622, UMC021, PIO20569, PIO1537, PIO20708, UMC110, and BNL8.39;

determining the RFLP profiles of individual corn plants in a segregating population of corn plants with respect to the selected genetic marker(s); and

selecting individual plants which have RFLP profiles which most closely match the preferred profile.

25. A method according to Claim 9 for identifying individual corn plants which have the desired genotype at a genetic marker locus

associated in inbred performance with kernels per kernel row, comprising the steps of

constructing a preferred RFLP profile with respect to one or more genetic markers selected from BNL12.06, UMC061, BNL6.20, E8, PI020713, PI020725, UMC031, UMC043, PI020622, PI0612, PI01524, PI01518, PI020589, UMC065, PI01014, UMC062, BNL10.39, UMC081, CSS1, PI020554, and PI020626;

determining the RFLP profiles of individual corn plants in a segregating population of corn plants with respect to the selected genetic marker(s); and

selecting individual plants which have RFLP profiles which most closely match the preferred profile.

26. A method according to Claim 9 for identifying individual corn plants which have the desired genotype at a genetic marker locus associated in inbred performance with number of kernel rows per ear, comprising the steps of

constructing a preferred RFLP profile with respect to one or more genetic markers selected from PI020690, UMC999, PI020726, UMC031, BNL5.46, PI020597, UMC042, UMC019, BNL7.65, UMC015, PI02071, PI01025, PI020608, PI0612, PI01524, PI01518, PI020589, PI020531, PI020566, PI02026P, PI02045, UMC065, UMC046, PI020569, PI01537, PI020708, PI020684, PI020746, UMC116, UMC110, BNL8.32, BNL8.44, PI0105, UMC057, and UMC044;

determining the RFLP profiles of individual corn plants in a segregating population of corn plants with respect to the selected genetic marker(s); and

selecting individual plants which have RFLP profiles which most closely match the preferred profile.

27. A method according to Claim 9 for identifying individual corn plants which have the desired genotype at a genetic marker locus

associated in inbred performance with kernel width, comprising the steps of

constructing a preferred RFLP profile with respect to one or more genetic markers selected from BNL12.06, UMC023, PI020726, UMC015, PI02071, PI020608, PI0612, PI01524, PI020589, PI020531, PI020566, PI02026P, PI02045, UMC065, PI020569, PI01537, PI020708, PI020684, PI020746, UMC116, UMC110, BNL8.32, BNL8.44, UMC012, UMC057, BNL10.13, and UMC044;

determining the RFLP profiles of individual corn plants in a segregating population of corn plants with respect to the selected genetic marker(s); and

selecting individual plants which have RFLP profiles which most closely match the preferred profile.

28. A method according to Claim 9 for identifying individual corn plants which have the desired genotype at a genetic marker locus associated in inbred performance with percent moisture at harvest, comprising the steps of

constructing a preferred RFLP profile with respect to one or more genetic markers selected from UMC094, PI020537, PI020603, PI020689, PI020640, PI020575, PI02044, PI020661, PI020518, BNL8.29, PI020557, UMC061, UMC043, PI020622, PI020531, UMC021, PI01016, BNL8.32, PI020593, BNL10.39, PI065, PI01513, UMC057, BNL10.13, and UMC044;

determining the RFLP profiles of individual corn plants in a segregating population of corn plants with respect to the selected genetic marker(s); and

selecting individual plants which have RFLP profiles which most closely match the preferred profile.

29. A method according to Claim 9 for identifying individual corn plants which have the desired genotype at a genetic marker locus

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associated in inbred performance with the ratio of yield in bushels per acre to percent moisture at harvest, comprising the steps of

constructing a preferred RFLP profile with respect to one or more genetic markers selected from BNL12.06, BNL5.59, UMCO61, BNL6.20, BNL6.16, UMCO42, BNL7.65, PIO20608, UMCO43, PIO20622, PIO612, PIO1524, PIO1518, PIO20589, PIO20527, PIO105, SH1, PIO20646, and PIO1513;

determining the RFLP profiles of individual corn plants in a segregating population of corn plants with respect to the selected genetic marker(s); and

selecting individual plants which have RFLP profiles which most closely match the preferred profile.

30. A method according to Claim 9 for identifying individual corn plants which have the desired genotype at a genetic marker locus associated in inbred performance with GDU shed, comprising the steps of

constructing a preferred RFLP profile with respect to one or more genetic markers selected from PIO20640, BNL12.06, UMCO34, PIO1012, PIO205, BNL5.37-, BNL5.37-, BNL10.24, PIO20713, PIO20725, BNL5.46, UMCO42, UMCO66, PIO20854, BNL13.05, BNL9.11, UMCO12, WX1, PIO2052, UMCO81, CSS1, PIO20554, BNL8.17, BNL7.21B, GLU1, PIO20646, PIO1513, PIO1033, UMCO57, and BNL10.13;

determining the RFLP profiles of individual corn plants in a segregating population of corn plants with respect to
the selected genetic marker(s); and

selecting individual plants which have RFLP profiles which most closely match the preferred profile.

31. A method according to Claim 9 for identifying individual corn plants which have the desired genotype at a genetic marker locus associated in inbred performance with GDU silk, comprising the steps of

constructing a preferred RFLP profile with respect to one or more genetic markers selected from PIO20690, PIO20537, PIO20640, BNL10.24, PIO20713, PIO20725, BNL5.46, UMC042, UMC066, PIO1524, PIO20854, BNL13.05, UMC012, PIO105, SH1, WX1, PIO2052, UMC081, CSS1, .PIO20554, BNL8.17, GLU1, PIO20646, PIO1513, and BNL10.13;

determining the RFLP profiles of individual corn plants in a segregating population of corn plants with respect to the selected genetic marker(s); and

selecting individual plants which have RFLP profiles which most closely match the preferred profile.

32. A method according to Claim 9 for identifying individual corn plants which have the desired genotype at a genetic marker locus associated in inbred performance with stay green, comprising the steps of

constructing a preferred RFLP profile with respect to one or more genetic markers selected from PIO20640, BNL12.06, PIO20654, BNL5.59, PIO20682, PIO20674, PIO20575, PIO20644, PIO2044, PIO20661, PIO20557, UMC034, BNL6.16, PIO1518, PIO20531, PIO20566, PIO20527, BNL8.39, BNL8.44, PIO2020, WX1, BNL8.17, UMC057, BNL10.13, and UMC044;

determining the RFLP profiles of individual corn plants in a segregating population of corn plants with respect to the selected genetic marker(s); and

selecting individual plants which have RFLP profiles which most closely match the preferred profile.

33. A method according to Claim 9 for identifying individual corn plants which have the desired genotype at a genetic marker locus associated in inbred performance with plant height, comprising the steps of

constructing a preferred RFLP profile with respect to one or more genetic markers selected from BNL12.06, UMC034, PIO1012, PIO205, PIO20576, PIO20508, BNL5.37-, BNL5.37-, BNL10.24, PIO20713, PIO20725,

PIO20728, BNL10.39, UMC120, UMC012, WX1, PIO2052, UMC081, PIO20554, PIO20626, PIO2075, GLU1, and PIO20646;

determining the RFLP profiles of individual corn plants in a segregating population of corn plants with respect to the selected genetic marker(s); and

selecting individual plants which have RFLP profiles which most closely match the preferred profile.

34. A method according to Claim 9 for identifying individual corn plants which have the desired genotype at a genetic marker locus associated in inbred performance with ear height, comprising the steps of

constructing a preferred RFLP profile with respect to one or more genetic markers selected from PIO20640, BNL12.06, PIO2044, UMC034, PIO1012, PIO205, PIO20576, PIO20509, PIO20508, PIO20508, BNL5.37-, BNL5.37-, BNL10.24, BNL6.16, PIO20521, UMC999, PIO20713, PIO20725, BNL6.29, PIO20728, BNL10.39, UMC120, UMC012, WX1, PIO2052, UMC081, PIO20554, and UMC057;

determining the RFLP profiles of individual corn plants in a segregating population of corn plants with respect to the selected genetic marker(s); and

selecting individual plants which have RFLP profiles which most closely match the preferred profile.

35. A method according to Claim 9 for identifying individual corn plants which have the desired genotype at a genetic marker locus associated in inbred performance with European corn borer second brood tolerance, comprising the steps of

constructing a preferred RFLP profile with respect to one or more genetic markers selected from BNL5.59, PIO20644, PIO205, BNL6.20, E8, BNL6.16, PIO20597, BNL15.45, UMC042, PIO20608, PIO612, PIO2045, UMC065, MDH2, PIO20595, PIO20599, PIO20569, PIO1537, PIO20708, UMC110, BNL10.39, UMC120, and PIO105;

determining the RFLP profiles of individual corn plants in a segregating population of corn plants with respect to the selected genetic marker(s); and

selecting individual plants which have RFLP profiles which most closely match the preferred profile.

36. A method according to Claim 9 for identifying individual corn plants which have the desired genotype at a genetic marker locus associated in inbred performance with root lodging, comprising the steps of

constructing a preferred RFLP profile with respect to one or more genetic markers selected from UMC094, PI020537, PI020603, PI020689, PI020640, PI020518, PI020576, PI020511, PI020558, PI020509, PI020508, PI020508, BNL5.37-, BNL10.24, PI020713, PI020725, UMC031, UMC066, UMC019, UMC065, MDH2, PI020595, and UMC081;

determining the RFLP profiles of individual corn plants in a segregating population of corn plants with respect to the selected genetic marker(s); and

selecting individual plants which have RFLP profiles which most closely match the preferred profile.

37. A method according to Claim 9 for identifying individual corn plants which have the desired genotype at a genetic marker locus associated in inbred performance with statistically transformed root lodging score, comprising the steps of

constructing a preferred RFLP profile with respect to one or more genetic markers selected from PI020640, PI020654, PI020644, PI020518, PI020576, PI020511, PI020558, PI020509, PI020508, PI020508, BNL5.37-, BNL5.37-, BNL10.24, UMC019, BNL6.29, UMC065, PI020581, and UMC081;

determining the RFLP profiles of individual corn plants in a segregating population of corn plants with respect to the selected genetic marker(s); and

selecting individual plants which have RFLP profiles which most closely match the preferred profile.

38. A method according to Claim 9 for identifying individual corn plants which have the desired genotype at a genetic marker locus associated in inbred performance with stalk lodging, comprising the steps of

constructing a preferred RFLP profile with respect to one or more genetic markers selected from BNL6.20, PIO20597, MDH2, PIO20595, PIO20599, PIO20728, UMC012, PIO105, PIO2075, and UMC057;

determining the RFLP profiles of individual corn plants in a segregating population of corn plants with respect to the selected genetic marker(s); and

selecting individual plants which have RFLP profiles which most closely match the preferred profile.

39. A method according to Claim 9 for identifying individual corn plants which have the desired genotype at a genetic marker locus associated in inbred performance with statistically transformed stalk lodging score, comprising the steps of

constructing a preferred RFLP profile with respect to one or more genetic markers selected from PIO20682, PIO20661, PIO20668, AMP1, BNL6.20, UMC042, PIO67, PIO20595, PIO20599, BNL13.05, PIO105, SH1, and BNL3.04;

determining the RFLP profiles of individual corn plants in a segregating population of corn plants with respect to the selected genetic marker(s); and

selecting individual plants which have RFLP profiles which most closely match the preferred profile.

40. A method according to Claim 9 for identifying individual corn plants which have the desired genotype at a genetic marker locus

associated in inbred performance with grain quality, comprising the steps of

constructing a preferred RFLP profile with respect to one or more genetic markers selected from BNL5.59, PIO20575, PIO20644, PIO2044, PIO20661, AMP1, PIO20557, UMCO61, PIO1012, PIO205, PIO2017, E8, PIO20509, PIO2071, PIO1025, BNL8.33, PIO20569, PIO1537, PIO20708, PIO20684, PIO20746, UMC116, UMC110, BNL8.32, BNL8.39, PIO20593, PIO1033, UMCO57, and BNL10.13;

determining the RFLP profiles of individual corn plants in a segregating population of corn plants with respect to the selected genetic marker(s); and

selecting individual plants which have RFLP profiles which most closely match the preferred profile.

41. A method according to Claim 9 for identifying individual corn plants which have the desired genotype at a genetic marker locus associated in hybrid performance with yield in bushels per acre, comprising the steps of

constructing a preferred RFLP profile with respect to one or more genetic markers selected from BNL6.20, PIO20576, PIO20558, PIO20509, PIO20508, PIO20508, BNL5.37-, BNL5.37-, BNL10.24, BNL6.16, BNL7.65, UMCO15, PIO2071, PIO20589, PIO20569, PIO1537, PIO20708, PIO20684, PIO20746, UMC116, UMC110, BNL8.32, BNL8.39, PIO20593, and PIO20714;

determining the RFLP profiles of individual corn plants in a segregating population of corn plants with respect to the selected genetic marker(s); and

selecting individual plants which have RFLP profiles which most closely match the preferred profile.

42. A method according to Claim 9 for identifying individual corn plants which have the desired genotype at a genetic marker locus associated in hybrid performance with % Moisture, comprising the steps of

constructing a preferred RFLP profile with respect to one or more genetic markers selected from PIO20640, BNL12.06, UMC061, UMC034, PIO1012, PIO205, PIO2017, BNL6.20, PIO20569, PIO1537, UMC116, BNL8.32, BNL10.39, UMC120, PIO20714, UMC012, PIO2052, PIO20646, and PIO1513;

determining the RFLP profiles of individual corn plants in a segregating population of corn plants with respect to the selected genetic marker(s); and

selecting individual plants which have RFLP profiles which most closely match the preferred profile.

43. A method according to Claim 9 for identifying individual corn plants which have the desired genotype at a genetic marker locus associated in hybrid performance with Bulk Density, adjusted, comprising the steps of

constructing a preferred RFLP profile with respect to one or more genetic markers selected from PIO20518, PIO1012, PIO205, PIO2017, BNL6.20, PIO20511, PIO20558, PIO20509, PIO20508, PIO20508, BNL7.65, UMC015, PIO2071, PIO1025, PIO612, PIO1524, PIO20589, PIO2045, PIO20569, PIO1537, PIO20708, PIO20684, PIO20746, UMC116, UMC110, BNL8.32, SH1, WX1, PIO2052, UMC081, CSS1, PIO20626, PIO2075, BNL3.04, PIO65, GLU1, PIO20646, and PIO1033;

determining the RFLP profiles of individual corn plants in a segregating population of corn plants with respect to the selected genetic marker(s); and

selecting individual plants which have RFLP profiles which most closely match the preferred profile.

44. A method according to Claim 9 for identifying individual corn plants which have the desired genotype at a genetic marker locus associated in hybrid performance with Yield to % moisture ratio, comprising the steps of

constructing a preferred RFLP profile with respect to one or more genetic markers selected from BNL12.06, PIO20518, BNL8.29, UMC061,

UMC034, PIO1012, PIO20576, PIO20558, PIO20509, PIO20508, PIO20508, BNL5.37-, BNL5.37-, BNL10.24, BNL6.16, UMC066, UMC019, BNL7.65, PIO2071, BNL8.33, UMC043, PIO20622, PIO612, PIO1524, PIO20589, and PIO2026P;

determining the RFLP profiles of individual corn plants in a segregating population of corn plants with respect to the selected genetic marker(s); and

selecting individual plants which have RFLP profiles which most closely match the preferred profile.

45. A method according to Claim 9 for identifying individual corn plants which have the desired genotype at a genetic marker locus associated in hybrid performance with GDU Shed, comprising the steps of

constructing a preferred RFLP profile with respect to one or more genetic markers selected from PIO1012, PIO205, PIO2017, PIO20576, PIO20558, PIO20509, PIO20508, PIO20508, BNL5.37-, BNL5.37-, BNL10.24, PIO20521, PIO1533, UMC999, PIO20713, PIO20725, BNL5.46, PIO612, PIO1524, PIO20589, BNL13.05, PIO1040, BNL10.39, UMC120, PIO20714, UMC012, WX1, PIO2052, UMC081, PIO20554, and BNL10.13;

determining the RFLP profiles of individual corn plants in a segregating population of corn plants with respect to the selected genetic marker(s); and

selecting individual plants which have RFLP profiles which most closely match the preferred profile.

46. A method according to Claim 9 for identifying individual corn plants which have the desired genotype at a genetic marker locus associated in hybrid performance with Stay green, comprising the steps of

constructing a preferred RFLP profile with respect to one or more genetic markers selected from PIO20690, PIO2017, BNL6.20, BNL5.37-, BNL10.24, UMC046, MDH2, PIO20595, PIO20599, PIO20569, PIO1537,

PIO20684, UMC116, BNL8.32, BNL10.39, UMC120, PIO20714, UMCO12, SH1, and BNL3.04;

determining the RFLP profiles of individual corn plants in a segregating population of corn plants with respect to the selected genetic marker(s); and

selecting individual plants which have RFLP profiles which most closely match the preferred profile.

47. A method according to Claim 9 for identifying individual corn plants which have the desired genotype at a genetic marker locus associated in hybrid performance with Dropped Ears, comprising the steps of

constructing a preferred RFLP profile with respect to one or more genetic markers selected from BNL10.39, UMCO81, BNL7.21B, and BNL10.13;

determining the RFLP profiles of individual corn plants in a segregating population of corn plants with respect to the selected genetic marker(s); and

selecting individual plants which have RFLP profiles which most closely match the preferred profile.

48. A method according to Claim 9 for identifying individual corn plants which have the desired genotype at a genetic marker locus associated in hybrid performance with Dropped Ears, Statistically transformed, comprising the steps of

constructing a preferred RFLP profile with respect to the genetic marker PIO20690;

determining the RFLP profiles of individual corn plants in a segregating population of corn plants with respect to the genetic marker; and

selecting individual plants which have RFLP profiles which most closely match the preferred profile.

49. A method according to Claim 9 for identifying individual corn plants which have the desired genotype at a genetic marker locus associated in hybrid performance with Ear height, comprising the steps of

constructing a preferred RFLP profile with respect to one or more genetic markers selected from PIO20640, UMCO34, PIO20576, PIO20511, PIO20558, PIO20509, PIO20508, PIO20508, BNL5.37-, BNL5.37-, BNL10.24, PIO20521, PIO1533, PIO20713, PIO20725, BNL8.33, PIO20569, BNL8.32, BNL8.39, PIO20593, BNL10.39, UMC120, PIO20714, UMC012, WX1, UMC081, PIO20646, and PIO1513;

determining the RFLP profiles of individual corn plants in a segregating population of corn plants with respect to the selected genetic marker(s); and

selecting individual plants which have RFLP profiles which most closely match the preferred profile.

50. A method according to Claim 9 for identifying individual corn plants which have the desired genotype at a genetic marker locus associated in hybrid performance with Plant height, comprising the steps of

constructing a preferred RFLP profile with respect to one or more genetic markers selected from PIO20690, PIO20668, PIO20518, PIO20576, PIO20511, PIO20558, PIO20509, PIO20508, PIO20508, BNL5.37-, BNL5.37-, BNL10.24, BNL6.16, PIO20521, PIO1533, UMC999, PIO1080, BNL8.33, PIO1040, BNL10.39, UMC120, UMC012, PIO65, GLU1, PIO20646, PIO1513, and UMC044;

determining the RFLP profiles of individual corn plants in a segregating population of corn plants with respect to the selected genetic marker(s); and

selecting individual plants which have RFLP profiles which most closely match the preferred profile.

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51.   A method according to Claim 9 for identifying individual
corn plants which have the desired genotype at a genetic marker locus
associated in hybrid performance with Grain quality, comprising the
steps of

constructing a preferred RFLP profile with respect to one or more
genetic markers selected from BNL12.06, BNL8.29, BNL6.20, UMC999,
BNL6.29, PIO20528, PIO20599, and BNL14.28;

determining the RFLP profiles of individual corn plants in a
segregating population of corn plants with respect to the selected
genetic marker(s); and

selecting individual plants which have RFLP profiles which most
closely match the preferred profile.

52.   A method according to Claim 9 for identifying individual
corn plants which have the desired genotype at a genetic marker locus
associated in hybrid performance with European Corn Borer Second Brood
Tolerance, comprising the steps of

constructing a preferred RFLP profile with respect to one or more
genetic markers selected from PIO2017, PIO20576, PIO20509, PIO20508,
BNL5.37-, PIO20569, PIO1537, PIO20708, UMC116, BNL8.39, PIO20593,
BNL10.39, UMC120, PIO20626, and PIO2075;

determining the RFLP profiles of individual corn plants in a
segregating population of corn plants with respect to the selected
genetic marker(s); and

selecting individual .plants which have RFLP profiles which most
closely match the preferred profile.

53.   A method according to Claim 9 for identifying individual
corn plants which have the desired genotype at a genetic marker locus
associated in hybrid performance with Root lodging, comprising the
steps of

constructing a preferred RFLP profile with respect to one or more
genetic markers selected from UMC094, PIO20511, BNL5.37-, BNL5.37-,

BNL10.24, BNL6.16, UMC999, BNL8.23, BNL6.29, PIO20527, PIO20528, PIO20854, PIO2045, PIO67, UMC065, MDH2, PIO20595, UMC012, PIO20562, and PIO20646;

determining the RFLP profiles of individual corn plants in a segregating population of corn plants with respect to the selected genetic marker(s); and

selecting individual plants which have RFLP profiles which most closely match the preferred profile.

54. A method according to Claim 9 for identifying individual corn plants which have the desired genotype at a genetic marker locus associated in hybrid performance with Root lodging, statistically transformed, comprising the steps of

constructing a preferred RFLP profile with respect to one or more genetic markers selected from UMC094, PIO20576, PIO20511, BNL5.37-, BNL5.37-, BNL10.24, UMC999, UMC019, BNL6.29, PIO20527, PIO20528, PIO20854, PIO2045, PIO67, UMC065, MDH2, PIO20595, UMC012, and PIO20562;

determining the RFLP profiles of individual corn plants in a segregating population of corn plants with respect to the selected genetic marker(s); and

selecting individual plants which have RFLP profiles which most closely match the preferred profile.

55. A method according to Claim 9 for identifying individual corn plants which have the desired genotype at a genetic marker locus associated in hybrid performance with Stalk lodging, comprising the steps of

constructing a preferred RFLP profile with respect to one or more genetic markers selected from PIO20622, PIO1537, BNL14.28, PIO20562, UMC057, and UMC044;

determining the RFLP profiles of individual corn plants in a

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segregating population of corn plants with respect to the selected genetic marker(s); and

selecting individual plants which have RFLP profiles which most closely match the preferred profile.

56. A method according to Claim 9 for identifying individual corn plants which have the desired genotype at a genetic marker locus associated in hybrid performance with Stalk lodging, statistically transformed, comprising the steps of

constructing a preferred RFLP profile with respect to one or more genetic markers selected from PIO20537, PIO1012, PIO205, E8, PIO20558, PIO20597, PIO20622, PIO612, PIO1524, UMC065, PIO1537, UMC110, PIO20626, PIO2075, PIO20646, PIO1513, UMC057, BNL10.13, and UMC044;

determining the RFLP profiles of individual corn plants in a segregating population of corn plants with respect to the selected genetic marker(s); and

selecting individual plants which have RFLP profiles which most closely match the preferred profile.

INTERNATIONAL SEARCH REPORT

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) 6

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(4):C12N 15/00,C07H 15/12, A01H 1/00, A01H 1/04
US CL: 435/172.3, 435/320, 536/27, 47/58, 800/1

II. FIELDS SEARCHED

Minimum Documentation Searched 7

Classification System	Classification Symbols
U.S.	435/172.3, 435/320, 536/27, 47/58, 800/1

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched 8

Databases: Chemical Abstracts Services Online (File CA,
1967-1989; File Biosis, 1969-1989). Automated Patent
System (File USPAT, 1975-1989). SEE ATTACHMENT.

III. DOCUMENTS CONSIDERED TO BE RELEVANT 9

Category *	Citation of Document, 11 with indication, where appropriate, of the relevant passages 12	Relevant to Claim No. 13
Y	Trends in Genetics, Volume 3, issued August 1987, (Amsterdam, Netherlands), Helentjaris "A genetic linkage map for maize based on RFLPS," pages 217-221, see page 221 in particular.	2-8,10-56
X		1 & 9
Y	Plant Molecular Biology, volume 5, issued 1985, (The Hague, Netherlands) Helentjaris et al., "Restriction fragment polymorphism as probes for plant diversity and there development as tools for applied plant breeding," see pages 109-118.	2-8,10-56
X		1 & 9
Y	Crop science, Volume 27, issued July-August 1987, (Madison, USA) Nienhuis et al., "Restriction fragment length polymorphism analysis of loci associated with insect resistance in tomato, "see pages 797-803.	1-56

* Special categories of cited documents: 10

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

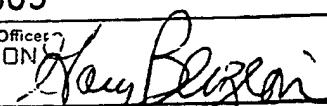"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
MAY 5, 1989	12 JUN 1989

International Searching Authority	Signature of Authorized Officer
ISA/US	GARY BENZION

Attachment To Form PCT/ISA/210
II. FIELDS SEARCHED

Dialog Information Services Inc., (File Agri).

Search Terms:

Plant Breeding, RFLP, Restriction length fragment poly-
morphism, correlation, maize, corn, zea, dropped ear
count, cold germination, soak germination, early stand
count, ear circumference, ear diameter, ear length,
yield, correlated response, cob circumference, cob
diameter, bare tip, kernel row length, kernel depth,
kernel per row, percent moisture, GDU shed, stay green,
European corn borer second brood, root lodging, stalk
lodging, inventor's names.

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

Y	Crop Science, Volume 27, issued July-August 1987 (Madison, USA) Stuber et al., "Molecular marker-facilitated investigations of quantitative loci in maize. II. Factors influencing yield and its component traits," See pages 639-649.	1-56
Y	Theoretical Applied Genetics, Volume 67, issued 1983. (New York, USA) Soller et al., "Genetic polymorphism in varietal identification and genetic improvement," see pages 25, 27-30, and 32.	1-56

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers   . because they relate to subject matter [12] not required to be searched by this Authority, namely:

2. ☐ Claim numbers   . because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [13], specifically:

3. ☐ Claim numbers_____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☒ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (supplemental sheet (2) (Rev. 11-87)

III. DOCUMENTS CONSIDERED TO BE RELEVANT   (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	Landry et al., Methods and applications of	2-8, 10-56
X	restriction length fragment polymorphism analysis to plants," in Bruening et al. eds., Tailoring genes for crop improvement. An agricultural perspective, published 1987, Press, (New York, USA). See pages 25-44 in particular pages 34-35.	1 & 9
Y	Burr et al., The application of restriction fragment length polymorphism to plant breeding in Setklow et al. eds., Genetic Engineering, Principles and Methods, published 1983, by Plenum Press (New York, USA), see pages 51-56.	1-56